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Stability of pO_2, pCO_2 and pH in Heparinized Whole Blood Samples: Influence of Storage Temperature with Regard to Leukocyte Count and Syringe Material

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Summary: Influences of storage temperature and blood cell metabolism in different types of syringes were investigated. Experiments were performed on blood samples with normal and elevated leukocyte counts. After equilibration with gas mixtures at normal pO_2 (86 mm Hg/11.5 kPa) and elevated pO_2 (140 mm Hg/18.7 kPa), sequential blood gas analyses were done within one hour. Storage temperatures were 4 °C or 22 °C.

In the first group of experiments we compared glass samplers with plastic syringes at different storage temperatures with regard to deviations of blood gas concentrations. The analysed samples had a normal cell count. Blood stored in glass syringes in ice water served as the reference, and it displayed virtually no changes. The deviations of pCO_2 and pH were relatively small. In plastic syringes the greatest increases for pO_2 occurred after storage at 4 °C, which can be explained by the increased solubility of oxygen and the higher O_2 affinity of haemoglobin at 4 °C. When stored at room temperature, the deviations in plastic syringes were smaller.

In a second group of experiments, the influence of cell metabolism was studied. Blood gases were analysed in samples with elevated leukocyte counts (20×10^9/l, 40×10^9/l, 60×10^9/l), and only glass syringes were used. It was demonstrated that after storage at 22 °C considerable losses in pO_2 occurred, while at 4 °C there was virtually no change. Deviations of pO_2, pCO_2 and pH are described in detail.

From the results, it is concluded that it is necessary to perform blood gas analysis within 15 minutes after sampling, when plastic syringes are used. These should not be chilled. When analysis cannot be done within 15 minutes, glass syringes have to be used, and these must be cooled. In severe leukocytosis ($\geq 40 \times 10^9$/l), glass syringes should be used in any case; these syringes should be stored in ice water until analysis.

Introduction

For convenience and for the reduction of infection hazards, glass syringes have been replaced widely by plastic containers for collecting blood gas samples. As blood gas values are extremely sensitive to preanalytical influences, handling of the sample as well as the type of syringe and the kind of heparin preparation must be controlled in order to obtain reliable results (1–9). The suitability of plastic syringes for blood gas sampling has been investigated often during the last two decades with controversial results (10–20). Against this background we felt it necessary to re-evaluate the suitability of plastic syringes for blood gas analysis. Data on syringe construction and material, blood handling and heparin composition were presented recently in this journal (21).

The present investigation has been focussed

(1) on the influence of storage temperature in different types of syringe (series I and II), and

(2) on errors caused by severe leukocytosis and thrombocytosis at 4 °C and 22 °C (series III–VII).

Materials and Methods

Syringes

- a glass syringe („G'" (Fortuna Optima®, Fa. Graf) as a „reference", which additionally was equipped with a metal platelet for mixing.
- a 1 ml-polypropylene syringe („B"), which was originally designed for insulin-application (Braun Omnifix®), and
- a commercial polypropylene blood-gas-sampler („R") (Radiometer QS90™).

The dead spaces of the G- and B-syringes were filled with an electrolyte-balanced heparin solution (Vetren®-200, Fa. Promonta). The Radiometer sampler is provided with a dry heparin preparation.

In series III – VIII we used glass syringes only.

Tonometry

Equilibration of heparinized blood from healthy individuals was performed with the thin-film-tonometer IL 237® (Instrumentation Laboratory), following the IFCC recommendation (22). The following gas mixtures were used:

- A: 0.1200 O_2, 0.0497 CO_2, N_2 balance (series I, III, V and VII)
- B: 0.1995 O_2, 0.0499 CO_2, N_2 balance (series II, IV, VI and VIII)

Measurements

pO_2, pCO_2 and pH were measured with a Corning C178® blood gas analyser. For quality control we used Confirm® (Ciba-Corning) at three levels daily.

For measurement of the cell count in the second group of experiments we used a Sysmex CC800® (Fa. Digitana).

Series of experiments

Series I and II

In this first two groups of experiments 8.5 ml of heparinized whole blood from healthy individuals were equilibrated for 20 min, then an aliquot was drawn anaerobically into the first syringe and measured immediately („0" min). Thereafter the syringe was stored either at ambient temperature (22 ± 1 °C) or in ice-water (4 °C). Subsequent syringes were treated in the same way. Each set consisted of three syringes, G, R and B, stored in iced water, and three stored at room temperature. After 30 and 60 min measurements were performed on each syringe. The sampling and measuring sequence for all syringes was identical in each set, but changed between sets to prevent any „position effects".

In series I, consisting of 30 sets of six syringes, measurements were done after equilibration with gas mixture „A".

Series II consisted of 20 sets of six syringes, which were measured after equilibration with gas mixture „B". The results are shown in figures 1 – 2.

Series III – VIII

For the second group of experiments, samples were prepared with white blood cell counts of 20×10^9/l, 40×10^9/l and 60×10^9/l and equilibrated as described. The alterations in pH and in blood gases were studied during storage in glass syringes at 4 °C or at 22 °C. The leukocyte-enriched samples used in this experiment were derived from stored blood units.

For transfusions, preparations of erythrocyte concentrates and plasma are needed. The remaining so called "buffy coat" is rich in leukocytes and platelets. Due to their immunological properties leukocytes are unwanted for transfusion purposes and so could be used for these experiments. By several low-speed centrifugation and separation steps it was possible to remove most of the interfering platelets and to prepare the above described blood samples with elevated white cell counts.

Each series consisted of 15 pairs of glass syringes, one of which was stored at ambient temperature while the other one was kept in ice-water.

The samples were anaerobically drawn out of the tonometer cuvette and measured as described above immediately, after 30 and 60 min storage.

Results

Series I and II

pO_2

As can be seen from figures 1 and 2, virtually no alterations occur in the iced glass syringes, which are superior to both cooled plastic syringes and to all uncooled syringes. In the uncooled glass syringe at the normal pO_2 level (pO_2 86 mm Hg/11.5 kPa, fig. 1), a slight decrease can be seen. In both plastic syringes at both temperatures there is a significant increase during the whole period of observation.

At the high level (pO_2 140 mm Hg/18.7 kPa, fig. 2), the decrease in the uncooled glass syringe is extreme, whereas the alterations in uncooled plastic syringes are relatively small after 30 and 60 min. After storing the samples in iced water, pO_2 sharply increases in both plastic syringes after 30 min and 60 min, the alterations being highly significant ($p \leq 0.001$) (fig. 1 – 2) and distinctly greater than at room temperature.

pCO_2

In the glass syringe the alterations of pCO_2 after storage in iced water are close to zero, whereas the average deviation after storage at ambient temperature is 1 – 2 mm Hg/0.13 – 0.26 kPa. In the Radiometer syringe, a surprising elevation of pCO_2 at both temperatures was found, which does not continue after 60 min of storage in ice-water (fig. 3). In the Braun syringe an insignificant tendency towards decreasing values can be seen at both temperatures.

pH

Slightly lower results after 30 min and 60 min were observed in all syringes after storage at 4 °C, whereas the decreases after 22 °C storage were markedly higher, the pH decreasing by between 0.01 and 0.029 after 60 min (fig. 4).

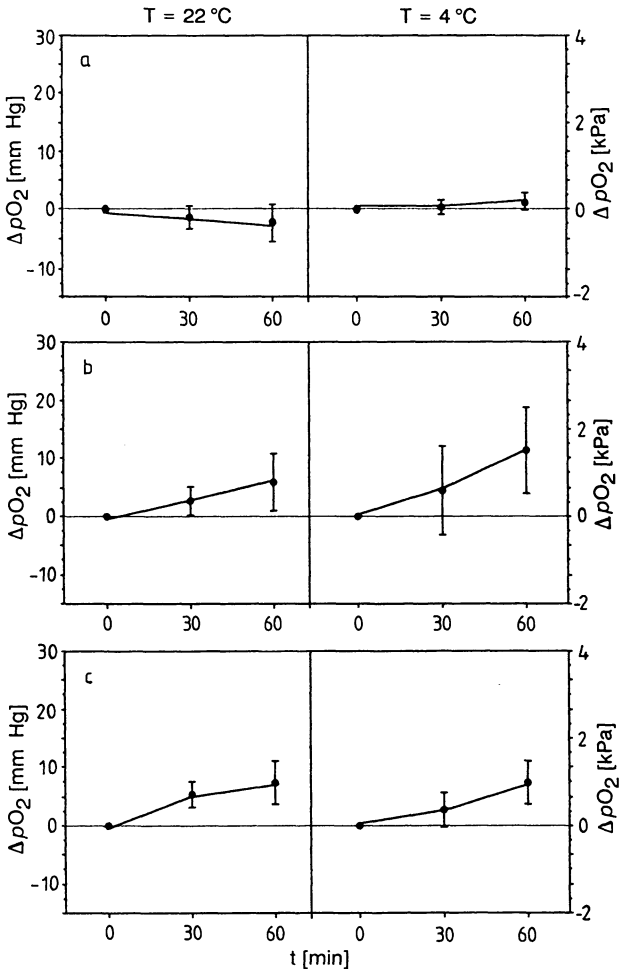

Fig. 1. Deviations of pO_2 dependent on storage temperature (left 22 °C, right 4 °C) after tonometry with pO_2 86 mm Hg/11.5 kPa
a) glass syringe n = 30
b) Radiometer syringe QS90™, n = 30
c) Braun 1 ml syringe „insulin type") n = 30

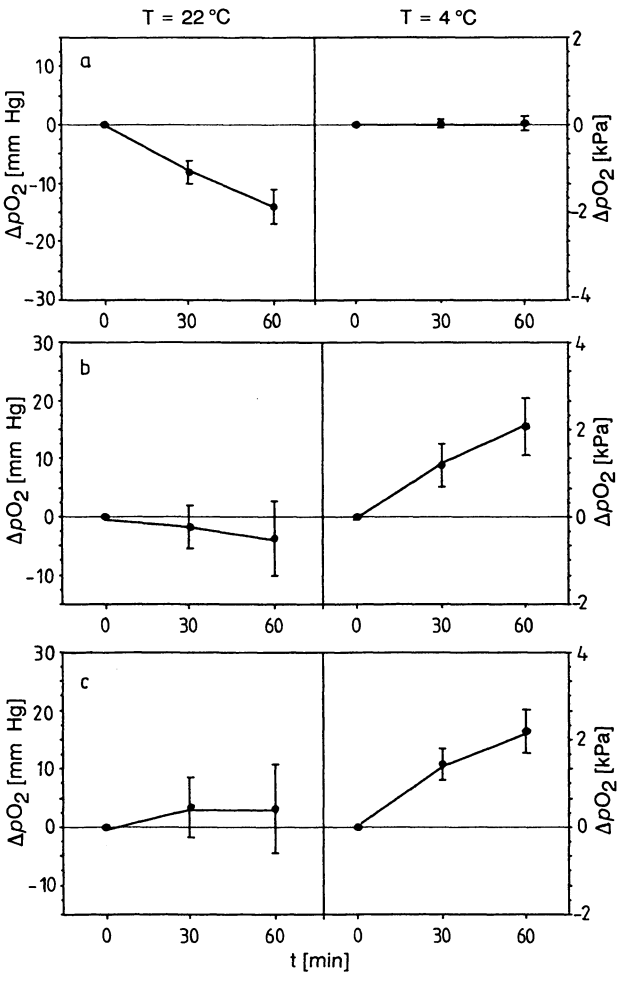

Fig. 2. Deviations of pO_2 dependent on storage temperature (left 22 °C, right 4 °C) after tonometry with pO_2 140 mmg Hg/18.7 kPa
a) glass syringe n = 20
b) Radiometer syringe QS90™ n = 20
c) Braun 1 ml syringe „insulin type") n = 20

Series III – VIII

pO_2

In all cooled samples, there were no significant changes in pO_2, which shows again the superiority of chilled glass syringes. We observed highly significant negative deviations in all series when they were stored at ambient temperature, ΔpO_2 varying from −11 mm Hg to −44 mm Hg (−1.46 to 5.87 kPa) after 60 min. The decrease was markedly higher in the samples with an initially higher pO_2 of about 140 mm Hg. The deviations increased with the increasing number of leukocytes, but there was no linear dependency between the cell count and ΔpO_2 (for details see figs. 5–6).

pCO_2

Corresponding to the decrease of pO_2, increases of pCO_2 in the unchilled samples of all series were seen, the total increase ranging from 1.6 mm Hg to 3.4 mm Hg (0.21 to 0.59 kPa) after 60 min. All results were significant at $p \leq 0.01$ or $p \leq 0.001$. We saw no remarkable change in the results for chilled syringes, the maximum change being less than 1 mm Hg/0.13 kPa.

pH

After 60 min storage at 4 °C the peak change was −0.007, which is clinically negligible. The samples stored at ambient temperature showed highly significant decreases ($p \leq 0.001$) in all samples after 30 min and 60 min with a maximum change of −0.036, which is nearly half of the reference interval.

Changes in calculated quantities

On the basis of the greatest mean increase in pCO_2 and in pH after 60 min storage at ambient tempera-

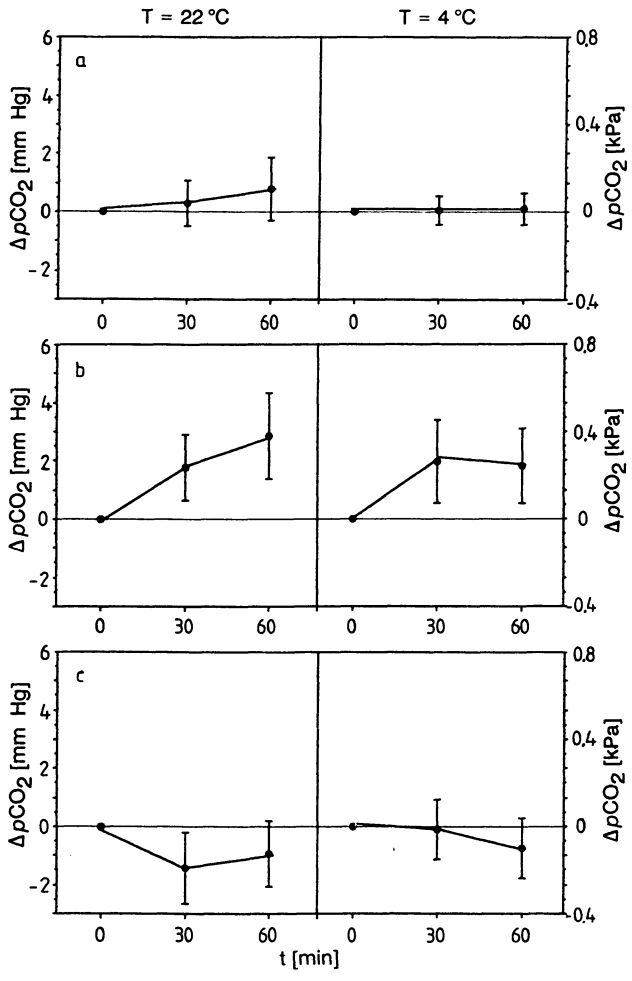

Fig. 3. Deviations of pCO_2 dependent on storage temperature (left 22 °C, right 4 °C) (pO_2 140 mm Hg/18.67 kPa)
a) glass syringe n = 20
b) Radiometer syringe QS90™ n = 20
c) Braun 1 ml syringe „insulin type") n = 20

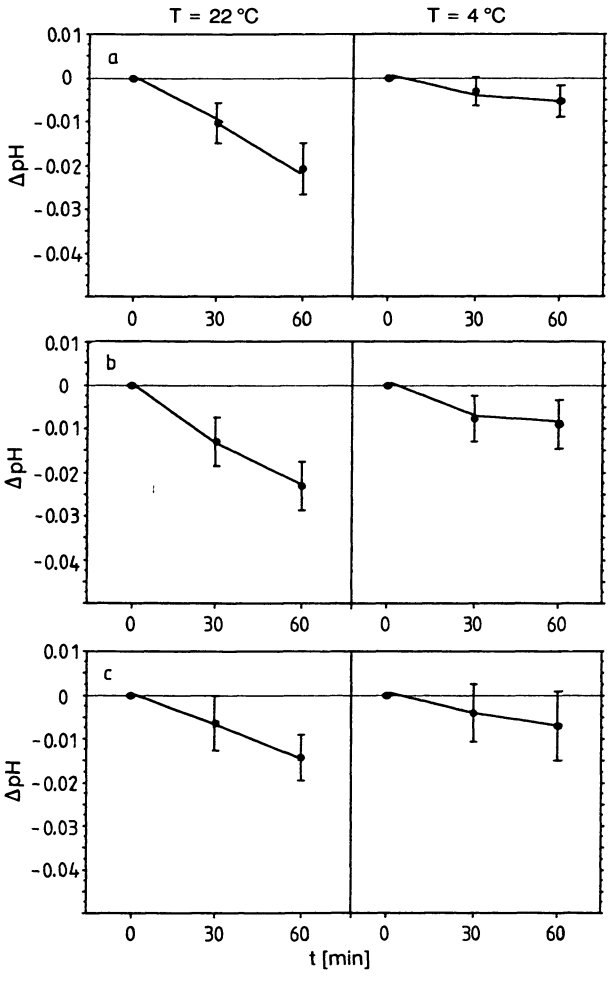

Fig. 4. Deviations of pH dependent on storage temperature (left 22 °C, right 4 °C) (pO_2 140 mm Hg/18.67 kPa)
a) glass syringe n = 20
b) Radiometer syringe QS90™ n = 20
c) Braun 1 ml syringe „insulin type") n = 20

ture, a decrease in $c_{HCO_3^-}$ of 0.02 mmol/l was calculated. This calculation demonstrates that the influence on the base quantities is negligible. Metabolic acid production is balanced by base release following deoxygenation.

Discussion

Efflux and/or influx of gases during storage is one of the preanalytical sources of error in blood gas analysis. Besides the permeability itself, the modulating influence of storage temperature on oxygen influx is important, as recently demonstrated by *Mahoney* et al. (23). A second source of error is the cell metabolism, which may alter the results to a great extent, depending on the storage temperature. We tried to find out whether there are significant changes of pO_2, pCO_2 and pH under different storage conditions, and in which direction the quantities might deviate during the time course of 60 min in different types of syringes.

Storage temperature effects

In order to minimize blood cell metabolism, it has become common practice to store samples in ice before blood gas analysis (24). In our experiments the effectiveness of this procedure for glass syringes was demonstrated. No relevant changes of pO_2, pCO_2 and pH could be detected up to 60 min storage in glass syringes at 4 °C.

pO_2

As plastic syringes are untight to gases, the results were expected to be different. This applies especially to pO_2. When samples are cooled from 37 °C to 4 °C, the solubility coefficient for pO_2 rises from 0.0214 to 0.0395 (25). Additional effects result from the decreasing $p50$, which indicates an increasing oxygen affinity of haemoglobin. In whole blood $p50$ decreases from 26.5 mm Hg/3.5 kPa to 4.5 mm Hg/0.61 kPa, when blood is cooled from 37 °C to 4 °C at a normal pH

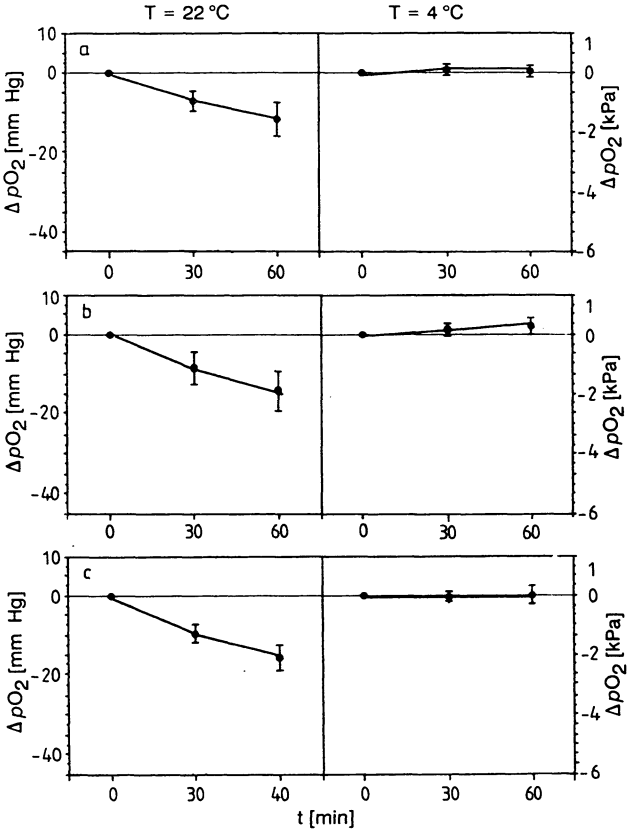

Fig. 5. Deviations of pO_2 in leukocyte-enriched samples dependent on storage temperature (left 22 °C, right 4 °C) after tonometry with pO_2 86 mm Hg/11.5 kPa (storage in glass syringes)
a) 20 × 10^9/l leukocytes, n = 15
b) 40 × 10^9/l leukocytes, n = 15
c) 60 × 10^9/l leukocytes, n = 15

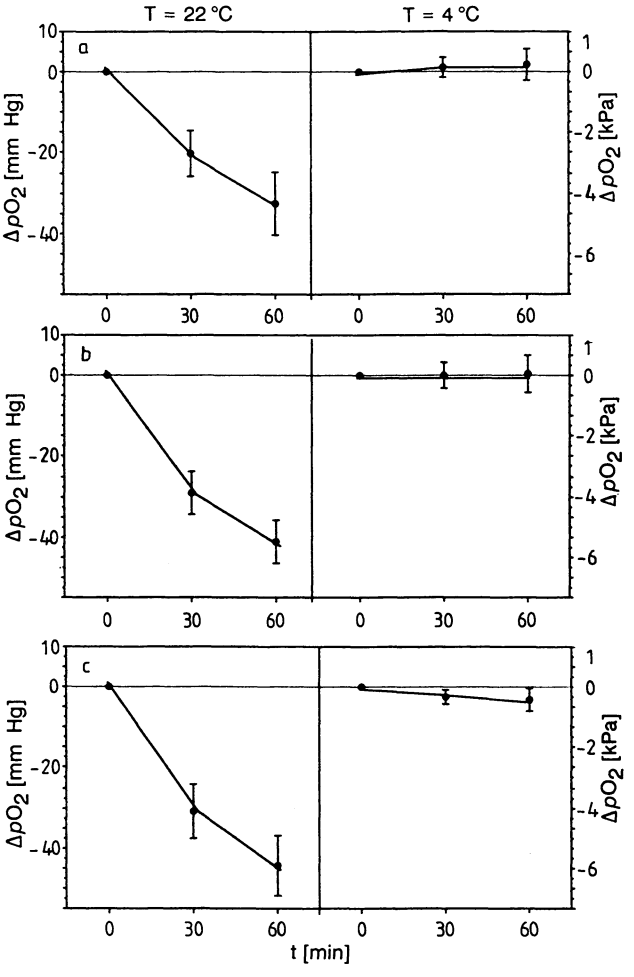

Fig. 6. Deviations of pO_2 in leukocyte-enriched samples dependent on storage temperature (left 22 °C, right 4 °C) after tonometry with pO_2 in 140 mm Hg/18.7 kPa (storage in glass syringes)
a) 20 × 10^9/l leukocytes, n = 15
b) 40 × 10^9/l leukocytes, n = 15
c) 60 × 10^9/l leukocytes, n = 15

(26). As a consequence, pO_2 in the sample will drop, and the gradient between sample and environment will rise. The result of cooling will be an influx of oxygen, as long as the pO_2 in the sample is lower than the ambient pO_2 of normally 150 mm Hg/20 kPa. When the sample is reheated for analysis, $p50$ and solubility will return to their original values at 37 °C, and the exogenous oxygen will be released, causing a falsely increased pO_2, especially at elevated pO_2, as shown in figure 2. In plastic syringes pO_2 remains more constant at room temperature than at 4 °C.

pCO_2 and pH

The characteristics of pCO_2 seem to be very similar when the plastic syringes are stored at room temperature or at 4 °C. An explanation for the surprising rise of pCO_2 in the Radiometer syringe is so far not available; the manufacturer has been informed. In all syringes the decrease in pH is markedly greater at room temperature, the greatest average deviation in samples with normal cell count being −0.023 after 60 min, which is as much as one fourth of the reference interval.

Leukocyte metabolism

When samples are stored at ambient temperature, the above changes will not be as great, but the pO_2 lowering influence of leukocytes and thrombocytes will become recognizable. The gas metabolism of these cells has been known for many years (27, 28, 29, 30). The resulting effects on blood gas values in leukaemia were first reported by *Bird* et al. (31), *Hess* et al. (32), *Laszlo* (33) and *Fox* et al. (34). But systematic efforts to quantify the effects of severe leukocytosis and/or thrombocytosis are lacking.

In our first group of experiments, using blood samples from healthy individuals unselected with regard to white cell count, the influence of metabolism on pO_2 seems to be partly balanced by leakage effects in both of the plastic syringes. In the second group of experiments, the effects of varying degrees of leukocytosis during storage of blood samples at room temperature

are demonstrated, i.e. remarkable decreases of pO_2 when the original pO_2 was normal, and extreme losses when the original pO_2 was elevated, which is not unusual in intensive care (figs 5–6). The increases in pCO_2 are sometimes remarkable (up to 3.4 mm Hg/ 0.6 kPa). The same applies to pH, with a maximum change of −0.036.

It should be emphazised that all deviations increase with the white cell count, but in our experiments they were not linearly dependent on the latter. This unexpected finding may be partly due to the metabolic influence of the remaining thrombocytes in the sample preparations; it was not possible to remove all thrombocytes without destroying the leukocytes, so that the residual thrombocyte count was 500 × 10^9/l to 800 × 10^9/l.

Conclusions

Summarizing the data of the present investigation it can be stated that

- the changes of pO_2 during storage in plastic syringes are distinctly smaller when they are stored at room temperature instead of ice-water. Referred to samples with original pO_2 of 86 mm Hg/11.5 kPa and 140 mm Hg /18.7 kPa, the deviations within 30 min seem to lie in the range −6 mm Hg/ −0.8 kPa to + 8 mm Hg/+ 1.07 kPa (in samples stored at 4 °C they range from + 6 mm Hg/+ 0.8 kPa to + 13.5 mm Hg/+ 1.8 kPa).
- Under the same conditions (plastic syringe at room temperature, 30 min) ΔpCO_2 varies from −3 mm Hg/−0.4 kPa to + 3 mm Hg/+ 0.4 kPa, with a clear dependence on the type of plastic syringe used.
- pH varies from −0.01 to −0.02.
- The alterations of pO_2 are much greater in the presence of leukocytosis and/or thrombocytosis. Blood cell metabolism then becomes the predominant influence on the result, leading to an extreme decrease of pO_2 during storage at room temperature.
- To keep the storage-dependent alterations safely within acceptable limits, namely

$$\Delta pO_2 \leq \pm 4 \text{ mm Hg/0.5 kPa}$$
$$\Delta pCO_2 \leq \pm 2 \text{ mm Hg/0.27 kPa}$$
$$\Delta pH \leq \pm 0.015,$$

it is necessary to restrict the storage interval for blood gas samples in plastic syringes to distinctly less than 30 min. A limit of 15 min storage at room temperature is recommended. This proposal is in accordance with the IFCC recommendation on whole blood collection, transport and storage for the determination of bloos gases and electrolytes (35), which is prepared by the Committee on pH, blood gases and electrolytes of the IFCC.

Recommendation

1. Blood gas samples in plastic syringes should be measured within 15 min. They should not be stored in iced water, but at room temperature.
2. Samples should be collected in glass syringes, when measurement cannot be guaranteed within 15 min. Glass syringes should be kept in ice-water until analysis.
3. If severe leukocytosis (≥ 40 × 10^9/l) and/or thrombocytosis (≥ 1000 × 10^9/l) is present, blood should always be collected in glass syringes and stored in ice-water. When, in this situation, the use of plastic syringes is unavoidable, the measurement must be performed immediately after sampling.

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